# COLUMN-CHROMATOGRAPHIC SEPARATION METHOD

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Description

Column-chromatographic separation method

The present invention relates to a column-chromatographic separation method using a liquid and low molecular weight fluid as eluant for the isolation and/or purification and/or preparative recovery of (natural) materials from mixtures, preferably mixtures of natural products.

Column-chromatographic separation methods are playing an increasing role in raw materials processing, in product purification and in chemical synthesis, both on a laboratory scale and in industrial-scale production. They are likewise indispensable in chemical analysis for the isolation, identification and quantitative/qualitative determination of individual components in mixtures. Further important applications for such separations are in recycling where poisons and pollutants, but also materials of value, are separated from waste/used materials such as wastewater and waste gases.

However, for cost reasons, only few column-chromatographic separation methods have been utilized commercially. Particularly for the purposes of preparative isolation of raw materials, in particular from mixtures of natural products, and in analysis thereof, column-chromatographic separations such as HPLC (high performance liquid chromatography) and SFC (supercritical fluid chromatography) are used.

HPLC makes use of the observation that the separation performance of a column increases with decreasing particle size of the stationary phase. HPLC employs a considerably finer chromatographic separation material (3-10 μm) than does gel chromatography (35-75 μm) or "classical" column chromatography (120-200 μm). The fineness of the separation materials does, however, require the use of high pressures (up to 40 MPa), which requires special engineering measures. The columns usually have a length of 5-100 cm (but usually up to 25 cm) and an internal diameter of 1-25 μm). When filled with silica gel or similar porous material (10 μm or less), a 25 cm long column can have 5 000 theoretical separation stages - even 65 000 theoretical separation stages/m can be achieved, depending on the stationary phase. Typical HPLC separations are carried out at room

temperature, preferably from 20 to 40°C (Merck, Chrombook, 1996, Darmstadt, Phenomenex, chromatography catalog, 99/00, Torrance, CA, USA). The pumps required in analytical HPLC can be operated at a pressure of up to 400 bar. In preparative HPLC, pumps are operated at up to a pressure of from 70 (pump output: 300 l/h) to 100 bar (pump output: 60 l/h of eluant (liquid)) (cf., for example, Merck, Chrombook, p. 233, 1996, Darmstadt). The limiting factor in HPLC is, particularly at high flows, the pump pressure. The columns used are therefore usually short: the smaller the particle diameter of the chromatographic material used, the shorter the columns have to be. In principle, this can be compensated by higher maximum working pressures, but this results in a tremendous increase in operating costs and can make the HPLC method uneconomical for preparative applications.

SFC has become established as a column-chromatographic separation method using gases ($CO_2$, $N_2O$, $SF_6$ etc.) in the supercritical state as mobile phase. In SFC, liquids and gases are heated under pressure and above the critical temperature and critical pressure in each case are converted into the supercritical state. In this state, supercritical fluids are distinguished from true liquids not only by their lower density, much lower viscosity and much higher diffusion coefficient but also, in particular, by their excellent solvent capability. For this reason, supercritical fluids comprising $CO_2$, ethylene, propane, ammonia, dinitrogen dioxide, water, toluene, nitrogen heterocycles, etc., are employed in extraction, known as supercritical fluid extraction (SFE or SCFE; also referred to as "destraction") of natural products. Known applications are, for example, the extraction of caffeine from coffee by means of supercritical $CO_2$, of bitter components of hops, fragrances, of hydrocarbons from petroleum or coal, for the reactivation of heterogeneous catalysts (cf. Wenclawiak (editor), Analysis with Supercritical Fluids: Extraction and Chromatography, Berlin: Springer 1992).

In general, SFC is carried out at temperatures above 35°C and a pressure on the column outlet side of above 120 bar.

Numerous separations at temperatures below the critical temperature of the mobile phase used have been described; cf. K. Anton, C. Berger (Ed.); Packed Columns, Marcel Dekker inc., New York, Basel, Hongkong, 1998.

The separation of mixtures of materials, in particular mixtures of natural products, by means of SFC is described in a series of publications, for example JP-A-10/316,991; Chirality (1992); 4(4), 252-62; J. chromatogr. (1989) 464(1), 125-37; Nihon Yukagakkaishi (1997), 46(11), 1335-1345; Semin. Food Anal. (1996), 1(2), 101-116; J. High Resolut. Chromatogr. (1996), 19(10), 569-570; Jasco Rep. (1991) 33(1), 6-11; Anal. Chem. (1990), 62(12), 394R-402R; and J. Chromatogr. Sci. (1990), 28(1). 9-14.

On page 64 (Anton supra), Vérillon and Coleman describe the temperature and pressure range for working with fluids such as $CO_2$ as from 3 to 200°C and 90-400 bar. The separations are carried out in the supercritical and subcritical region, but not in the liquid region i.e. in a pressure range below the critical pressure and temperature.

On page 409 in chapter 14 (supra Anton), Jusforques describes the working range required as being temperatures of from 0 to 150°C and pressures of from 100 to 350 bar (SFC apparatus: Super C12 for preparative SFC).

The company Prochrom likewise indicates a working pressure range of from 100 to 350 bar at temperatures of from 10 to 80°C (Prochrom, brochure on semipreparative supercritical fluid chromatograph, type: SuperC 20, 1998, Chamigneulle, France).

EP 0 099 765 B1 discloses that the inlet pressure of a mixture into the column has to be greater than the critical pressure of the eluant, and in the range from 1.05 to 3. Pressures lower than the critical pressure are expressly described as precipitation conditions.

In NO 163139, M. Perrut describes only the supercritical and subcritical range, namely pressures of 70-250 bar and temperatures of from 25 to 80°C, as separation conditions.

In the prior art, no chromatographic separations using mobile phases in a working range below the critical temperature and pressure are described.

Schultz et al. (Schultz, W.G.: Randall, J.M.: Food Technol. 24, 1282 (1970)) describe only an unspecific extraction at 22°C and 63 bar of flavor

concentrates from homogenized fruits and fruit juices. A chromatographic separation is not carried out.

It is thus an object of the present invention to provide a column-chromatographic method of the liquid chromatography type for the industrial isolation and/or purification and/or preparation recovery of (natural) materials from mixtures of (natural) materials, which leads to pure products as a result of a high separation performance.

The present invention accordingly provides a method for the column-chromatographic separation of mixtures of materials using a fluid or a mixture of fluids which are gaseous at 25°C and 1 bar in a liquid, nonsubcritical and noncritical state as eluant (hereinafter referred to as the method of the invention).

The eluants used according to the invention are low molecular weight compounds which are gaseous under normal conditions (25°C; 1 bar) and are used under such temperature and pressure conditions that they are in the liquid state but not in the critical state nor in the subcritical state (i.e. not above the critical temperature and below the critical pressure).

Preferred eluants for the method of the invention are volatile compounds having a low interaction.

Particularly preferred eluants are liquid dinitrogen oxide ($N_2O$); liquid fluorocarbons or fluorinated hydrocarbons ("Freons"), e.g. chlorotrifluoromethane ($CClF_3$), trifluoromethane ($CHF_3$), tetrafluoromethane ($CF_4$), 1,1,1,2-tetrafluoroethane ($CF_4H_2$), liquid carbon dioxide ($CO_2$), liquid sulfur hexafluoride ($SF_6$), liquid propene ($C_3H_6$), liquid propane ($C_3H_8$), liquid ammonia ($NH_3$), liquid sulfur dioxide ($SO_2$), liquid xenon (Xe), liquid ethane ($C_2H_6$), which are gaseous under normal conditions.

Preference is given to using eluants which have a dynamic viscosity of $10^{-4}$-$10^{-6}$ Pa s, preferably $2*10^{-4}$-$2*10^{-6}$ Pa s, a density of 0.5-1.2 g/ml, preferably 0.5-1.2 g/ml.

For the purposes of the present patent application, the dynamic viscosity is measured using a capillary viscometer at 25°C.

In the method of the invention, such lower molecules are present in liquid form as eluant in the mobile phase.

Preference is given to using those eluants which are known to a person skilled in the art of SFC.

However, liquid carbon dioxide is very particularly preferred and is employed in the liquid temperature range from -57°C to 31.3°C, preferably from 0 to 20°C.

Furthermore, liquid carbon dioxide is very particularly preferred and is employed in the pressure range above 30 bar, preferably from 30 to 150 bar and very preferably from 30 to 60 bar. The liquid pressure range of carbon dioxide is from 5.2 to 73.7 bar.

The critical pressure of carbon dioxide is 73.7 bar and its critical temperature is 31.3°C. The triple point of carbon dioxide is at -57°C and 5.2 bar.

Of course, the eluants mentioned can also be present in admixture.

The working range of the low molecular weight eluants used is the liquid phase at the associated p,T values.

These p,T values likewise prevail in the chosen chromatographic separation column through which the eluant is passed as mobile phase.

For the purposes of the present invention, a column-chromatographic separation method means that separation of materials occurs by partition, i.e. by dissolution in two, mutually immiscible phases, and by adsorption on a solid (adsorbent) in a stationary phase in the presence of an eluant as mobile phase. It is thus possible to refer to the method as liquid-solid chromatography (LSC). There are terminology recommendations by IUPAC for the terms utilized here, and these are expressly incorporated by reference.

In a particular embodiment, the mobile phase comprising an eluant is admixed with a modifier customary in chromatography, for example an

alcohol which is liquid under the operating conditions, e.g. ethanol or methanol. This modifier can, if desired, be admixed with further auxiliaries and additives such as acids or bases (e.g. acetic acid or diethylamine).

The method of the invention has numerous advantages compared to known methods such as HPLC or SFC methods.

A particular advantage compared to known HPLC or SFC methods is that it is possible to employ lower pressures, for example pressures of only up to about 70 bar, in the method of the invention. This enables cost savings since all components required, e.g. pumps, pressure sensors or pipes, can be designed accordingly or made smaller. Safety measures required for occupational hygiene and operational safety can be reduced.

It has surprisingly been found that the method of the invention can be applied in the presence of any commercial solid stationary phases used for the purposes of HPLC and/or SFC.

However, preference is given to columns containing modified or unmodified phases based on silica gel, aluminum oxide or titanium dioxide or supported polymeric stationary phases e.g. diol phase, aminopropyl phase, RP8 and RP18 phases applied to silica gel, aluminum oxide or titanium dioxide, with particular preference being given to these phases being present on silica gel supports.

The method of the invention can be employed for column lengths within a wide range. The columns are usually at least 10 cm long, but preference is given to lengths of 0.25-2.0 m, particularly preferably 1.1-1.7 m.

These longer columns ensure a high separation performance and can be achieved since the method of the invention causes no large pressure drop in the column.

The pressure drop can be estimated by means of formula 1 in table 1. Since the viscosity of the liquid eluants used is low, the resulting pressure drop is also small. As a result of the low pressure drop per cm of column, the column can be made correspondingly long at the same particle diameter of the stationary phase or, compared to HPLC, smaller particle

diameters can be used for a given column length.

The method of the invention can be operated using customary apparatuses as are also used in known SFC methods.

To explain the invention further, the operation of a customary SFC apparatus on an industrial scale using the eluant $CO_2$ is described below.

$CO_2$ to be fed in is taken from a liquid $CO_2$ reservoir and cooled by means of a heat exchanger. The $CO_2$ conveyed by the pump is brought to supercritical conditions by means of a heat exchanger ($T > 31°C$, $p >$ 74 bar), typically 40-60°C. This $CO_2$ flows through the separation column and is depressurized via a pressure regulating valve. This pressure regulating valve regulates the column outward pressure which prevails at the end of the separation column and is kept above the critical pressure ($p >$ 74 bar). This ensures supercritical separation conditions in the separation column. The $CO_2$ is cooled as a result of the adiabatic expansion at the pressure regulating valve. The $CO_2$ is subsequently heated by means of a heat exchanger and brought into the gaseous state. In a precipitator, the product is separated from the gaseous $CO_2$. The product is separated from the gaseous $CO_2$. The gaseous $CO_2$ goes via the heat exchanger, which liquefies it again, to the reservoir. A closed $CO_2$ circuit is thus obtained. The product is taken discontinuously from the stock container by means of the pump and is injected into the separation column. For this customary SFC arrangement, a total of at least four heat exchangers are necessary, which is very energy intensive.

In the context of the method of the invention, there are numerous possible functional arrangements in addition to the procedure described above for the SFC method, and some of these will be described below.

The particular advantage of the method of the invention is that energy-intensive alternate cooling and heating of the $CO_2$ is avoided.

$CO_2$ to be fed in is taken from a liquid $CO_2$ reservoir and cooled by means of a heat exchanger. The $CO_2$ conveyed by the pump is brought to the separation temperature by means of the heat exchanger. However, this can be very small since the $CO_2$ is only heated from typically 0-5°C to

5-20°C.

If low separation temperatures, e.g. 5°C, are chosen, this heat exchanger can also be taken out of operation. The liquid $CO_2$ flows through the separation column. By means of a switching valve, the individual fractions are diverted to fractionation columns, for example to three fractionation columns. Any number of fractionation columns are possible, but in the simplest case there is one fractionation column and one additional pipe which connects the switching valve to a further switching valve.

The column outward pressure is regulated by means of a pressure regulating valve. At this valve, the $CO_2$ is depressurized. After injection of the sample (assumption: the sample contains 3 components A, B and C), the sample is separated on the separation column.

During elution of component A, the switching valve is set so that $CO_2$ and component A is diverted in the direction of the further switching valve. The liquid $CO_2$ is depressurized. The $CO_2$ loses its solvent power. Component A is thus retained on the fractionation column. The $CO_2$ is cooled further (if necessary) by means of a heat exchanger and goes back to the reservoir.

After elution of component A from the column, the switching valve is switched over to a further fractionation column. The component B to be eluted is retained on this fractionation column. Component C is retained on a further fractionation column. When no sample substance is eluted, the $CO_2$ stream can be conveyed either via one of the fractionation columns or via an additional pipe which is provided with a pressure regulating valve and connects the switching valve to the further switching valve.

The chromatographic material of the separation column is chosen so that a maximum resolution of the sample components is achieved. The material of the fractionation columns is chosen so that maximum adsorption of the respective sample component is achieved under the conditions prevailing there during the separation.

This procedure can be repeated for a plurality of injections (until the capacity of a fractionation column has been reached). Elution of the

respective components is carried out using a suitable strong solvent, e.g. ethanol. A separate pump conveys the eluant via the switching valve to the outlet downstream of the further switching valve. The desired fractions are thus collected as solutions in liquid in this case.

The advantage of this apparatus described is that neither a fourth heat exchanger nor a precipitator is required.

In further embodiments of the method of the invention, the pressure regulating valve can be installed directly downstream of the separation column. The expanded $CO_2$ together with the respective sample component is conveyed only after depressurization by means of the switching valve to the appropriate fractionation column. The advantage over the above-described arrangement is that only one pressure regulating valve is required.

In further embodiments of the method of the invention, the pressure regulating valves can be installed downstream of the fractionation columns. In addition, further precipitators are located downstream thereof.

In this arrangement, the pressure is regulated downstream of the fractionation column. The material of the fractionation columns is selected so that the components A, B and C are retained in the appropriate fractionation column under the prevailing conditions. After repeated injection and collection of the sample components on the fractionation columns, the individual components can be eluted by increasing the elution strength of the $CO_2$ and/or adding a modifier and be separated from the $CO_2$ by means of the respective precipitator.

As an alternative, elution can also be carried out using a liquid eluant. In this case, the precipitators can be omitted. The apparatus can also be modified by using only one precipitator downstream of the switching valve instead of three precipitators.

A customary SFC unit can be operated much more efficiently in energy terms by means of the method of the invention. In the heat exchanger downstream of the reservoir, the $CO_2$ has to be heated to only a small degree, if at all. In addition, the lower pressure upstream of the pressure

regulating unit results in reduced cooling due to the adiabatic expansion and much less energy therefore has to be supplied to the heat exchanger downstream of the separation column. In principle, the method of the invention can be applied to any SFC unit.

The method of the invention is very particularly useful for the column-chromatographic separation of unsaturated, in particular polyunsaturated, higher fatty acids or derivatives thereof, e.g. esters or lipids, preferably from natural oils such as vegetable oil, alga oil, oil from microorganisms or fungi and also fish oil, and thus for the isolation and purification of SDA (stearidonic acid, 18:4, n3), AA (arachidonic acid, 20:4, n-6), ETA (eicosatetraenoic acid, 20:4, n-3), EPA 25 (eicosapentaenoic acid, 20:5, n-3), DPA (docosapentaenoic acid, 22:5, n-3), DHA (docosahexaenoic acid, 22:6, n-3), GLA (gamma-linolenic acid, 18:3, n-6), ALA (alpha-linolenic acid, 18:3, n-3), DPA (docosapentaenoic acid, 22:5, n-6).

The following examples serve to illustrate the invention without restricting it to these examples.

Example 1:

A customary SFC apparatus as described above was used. The columns used had a length of up to 170 cm. The working pressures normally employed were generally from 120 to 350 bar. These columns can be used in SFC since the pressure drops over a column of this length are only small (normally 20-50 bar). The low pressure drops make it possible to use higher flows which in SFC, in contrast to HPLC, lead to only a small decrease in efficiency. Carbon dioxide was used as eluant. The operating parameters are listed in table 1 below. In addition to the use according to the invention of liquid or fluid carbon dioxide, supercritical carbon dioxide was also used.

Table 1: Operating parameters

| Calculation p | Column: 101 | L(cm) | d(cm) | r(cm) | dp(µm) | | | Type |
|---|---|---|---|---|---|---|---|---|
| | | 110 | 9 | 4.5 | 25 | | | |
| | ρ(g/ml) | η dyn.(Pa s) | η dyn.(cpoise) | F(kg/h) | F(ml/min) | | Δp(bar) | |
| **80 bar, 50°C** | 0.219 | 2.05E-05 | 0.02053 | 300 | 22 831.05 | | **21.6** | SFC |
| **100 bar, 50°C,** | 0.3899 | 2.95E-05 | 0.02945 | 300 | 12 823.80 | | **17.4** | SFC |
| **200 bar, 50°C** | 0.784 | 6.80E-05 | 0.06797 | 300 | 6 377.55 | | **20.0** | SFC |
| **25°C, 150 bar** | 0.8771 | 8.63E-05 | 0.0863 | 300 | 5 700.60 | | **22.7** | SbFC |
| **0°C, 150 bar** | 1 | 1.23E-04 | 0.1234 | 300 | 5 000.00 | | **28.4** | SbFC |
| **0°C, 300 bar** | 1.055 | 1.47E-04 | 0.1466 | 300 | 4 739.34 | | **32.0** | SbFC |
| **0°C, 40 bar** | 0.9333 | 1.01E-04 | 0.1009 | 300 | 5 357.33 | | **24.9** | LFC |
| **10°C, 50 bar** | 0.8705 | 8.00E-05 | 0.08 | 300 | 5 743.83 | | **21.2** | LFC |
| **25°C, 70 bar** | 0.744 | 6.04E-05 | 0.06043 | 300 | 6 720.43 | | **18.7** | LFC |
| **MeOH, 20°C** | 0.79 | 5.84E-04 | 0.584 | 300 | 6 329.11 | | **170.4** | HPLC |
| **EtOH, 20°C** | 0.789 | 1.20E-03 | 1.201 | 300 | 6 337.14 | | **350.9** | HPLC |

Calculation according to: $\Delta p = \frac{F \cdot \eta \cdot L \cdot 1000}{60 \cdot \pi \cdot r^2 \cdot dp^2}$

where

| | | |
|---|---|---|
| 5 | $\Delta p$ | pressure drop in [bar] |
| | F | volume flow of the eluant in [ml/min] |
| | L | length of the column in [cm] |
| | $\eta$ | viscosity in [cpoise]; 1000 cpoise = 1 Pa s |
| | r | internal radius of the column in [cm] |
| 10 | Dp | particle diameter in [µm] |

Example 2:

Separations of an ethyl ester of fish oil on two different stationary phases were carried out. In each case, both supercritical conditions and also a temperature/flow combination in the range according to the invention were chosen. In table 2 below, the purities of the EPA and/or DHA of the fractions collected are compared with one another.

A fish oil was separated under various conditions: liquid phase (T = 18°C, $p_n$ - 52 bar; F = 300 kg/h); supercritical phase: (T = 46°C, $p_n$ = 105 bar; F = 300 kg/h); stationary phase: aminopropyl; mobile phase: $CO_2$.

The EPA content in % of the respective fraction is shown in table 2. It can easily be seen that the profile is very similar. However, the separation in the liquid range according to the invention was much quicker.

A reduction from about 75 min to only 40 min was possible. The absolute purity which could be achieved was somewhat lower (about 90% in the liquid range instead of 95% under supercritical separation conditions), but if the yield calculation is based on the fractions having an EPA purity above 80%, this corresponded to 52.7% of the mass injected in the supercritical range and 52.0% in the liquid range. These figures make it clear that the separations achieved are very similar.

However, the plants necessary for the liquid range are significantly cheaper since they only have to be suitable for pressures up to about 70 bar and do not have to be designed for up to 300 bar.

Table 2: EPA content in % in the case of separation using liquid carbon dioxide

| Separation time (min) | EPA content of the fraction (%) |
|---|---|
| 12 | 1.4 |
| 12.5 | 0.18 |
| 13 | 0.12 |
| 13.5 | 0.25 |
| 14 | 0.19 |
| 14.5 | 0.23 |
| 15 | 0.53 |
| 15.5 | 3.61 |
| 16 | 13.94 |
| 16.5 | 27.28 |
| 17 | 44.65 |
| 17.5 | 64.79 |
| 18 | 73.59 |
| 18.5 | 79.96 |
| 19 | 84.14 |
| 19.5 | 86.69 |
| 20.5 | 89.07 |
| 21 | 89.64 |
| 21.5 | 89.45 |
| 22 | 88.05 |
| 22.5 | 87.01 |
| 23 | 85.76 |
| 23.5 | 84.1 |
| 24 | 82.16 |
| 24.5 | 80.53 |
| 25 | 78.8 |
| 25.5 | 76.97 |
| 26 | 75.63 |
| 26.5 | 74.09 |
| 27 | 73.21 |
| 28.5 | 69.27 |
| 32 | 59.55 |
| 34 | 51.7 |
| 37 | 26.52 |
| 40 | 17.67 |

EPA content in % in the case of separation using supercritical carbon dioxide

| Separation time (min) | EPA content of the fraction (%) |
|---|---|
| 21 | 2.03 |
| 23 | 0.99 |
| 25 | 0.29 |
| 28 | 0.54 |
| 30 | 3.01 |
| 32 | 13 |
| 33 | 14.23 |
| 34 | 14.35 |
| 35 | 18.63 |
| 36 | 25.5 |
| 37 | 36.16 |
| 38 | 54.23 |
| 39 | 76.62 |
| 40 | 91.08 |
| 41 | 93.96 |
| 42 | 94.78 |
| 43 | 95.18 |
| 44 | 95.44 |
| 45 | 95.31 |
| 46 | 95.25 |
| 47 | 94.34 |
| 48 | 93.67 |
| 49 | 93.03 |
| 50 | 92.34 |
| 51 | 91.7 |
| 52 | 91.24 |
| 53 | 90.67 |
| 54 | 89.42 |
| 56 | 86.79 |
| 59 | 79.34 |
| 62 | 61.96 |
| 67 | 14.71 |
| 121 | 1.95 |

In table 3, the purities of the fractions in respect of both EPA and DHA are listed for the same separations. Here too, it can be seen that the separations are comparable, although the separation in the liquid range can be carried out in half the time using this phase.

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A fish oil was separated under various conditions: liquid phase (T = 18°C, pn = 52 bar; F = 300 kg/h); supercritical phase: (T = 46°C, pn = 105 bar; F = 300 kg/h); phase: aminopropyl; mobile phase: $CO_2$

Table 3: EPA/DPA content in % in the case of separation using liquid carbon dioxide and EPA/DPA content in % in the case of separation using supercritical carbon dioxide

| Separation time (min) | EPA/DPA content of the fraction (%) | Separation time (min) | EPA/DPA content of the fraction (%) |
|---|---|---|---|
| 12 | 1.4/2.99 | 21 | 2.03/85.53 |
| 12.5 | 0.18/0.31 | 23 | 0.99/9.92 |
| 14 | 0.12/0.18 | 25 | 0.29/0.65 |
| 13.5 | 0.25/0.32 | 28 | 0.54/0.27 |
| 14 | 0.19/0.26 | 30 | 3.01/0.37 |
| 14.5 | 0.23/0.27 | 32 | 13/0.28 |
| 15 | 0.53/0.12 | 33 | 14.23/02 |
| 15.5 | 3.61/0.16 | 34 | 14.35/0.01 |
| 16 | 13.94/0.01 | 35 | 18.63/0.01 |
| 16.5 | 27.28/0.11 | 36 | 25.5/0.15 |
| 17 | 44.65/0.01 | 37 | 36.16/0.01 |
| 17.5 | 64.79/0.01 | 38 | 54.23/0.01 |
| 18 | 73.59/0.01 | 39 | 76.62/0.22 |
| 18.5 | 79.96/0.01 | 40 | 91.08/0.01 |
| 19 | 84.14/0.01 | 41 | 93.96/0.01 |
| 19.5 | 86.69/0.01 | 42 | 94.78/0.01 |
| 20.5 | 89.07/0.32 | 43 | 95.18/0.13 |
| 21 | 89.64/0.75 | 44 | 95.44/0.01 |
| 21.5 | 89.45/1.41 | 45 | 95.31/0.27 |
| 22 | 88.05/2.58 | 46 | 95.25/0.35 |
| 22.5 | 87.01/3.72 | 47 | 94.34/0.44 |
| 23 | 85.76/5.15 | 48 | 93.67/0.58 |
| 23.5 | 84.1/6.66 | 49 | 93.03/0.72 |

| | | | |
|---|---|---|---|
| 24 | 82.16/8.14 | 50 | 92.34/0.93 |
| 24.5 | 80.53/10 | 51 | 91.7/1.2 |
| 25 | 78.8/11.66 | 52 | 91.24/1.48 |
| 25.5 | 76.97/13.17 | 53 | 90.67/1.88 |
| 26 | 75.63/14.81 | 54 | 89.42/2.43 |
| 26.5 | 74.09/16.12 | 56 | 86.79/3.65 |
| 27 | 73.21/17.31 | 59 | 79.34/8.53 |
| 28.5 | 69.27/21.06 | 62 | 61.96/21.54 |
| 32 | 59.55/28.9 | 67 | 14.71/62.52 |
| 34 | 51.7/35.27 | 121 | 1.95/81.27 |
| 37 | 26.52/54.38 | | |
| 40 | 17.67/62.17 | | |

Tables 4 and 5 show the results of a separation of the same starting material on a different stationary phase. Here too, it can be seen that the separations under the two conditions are very similar. On this stationary phase, however, the substances elute later under liquid conditions. The maximum EPA purity which can be achieved on this stationary phase is about 80%. If, for comparison, an EPA purity of more than 70% is defined for the mass yield, the yield of 36.2% under liquid conditions is significantly higher than in the case of supercritical conditions where it is only 30.3%. The strongly pronounced tailing of both of the EPA and the DHA is presumably attributable to silanol groups which are still highly active. This phase is less suitable for the separation. However, it was able to be shown that the separations in the various states are comparable.

A fish oil was separated under different conditions: liquid phase (T = 18°C, pn = 52 bar; F = 300 kg/h); supercritical phase: (T = 46°C, pn = 160 bar; F = 300 kg/h); stationary phase: silica gel; mobile phase: $CO_2$.

Table 4:

| EPA content in % in the case of separation using liquid carbon dioxide | | EPA content in % in the case of separation using supercritical carbon dioxide | |
|---|---|---|---|
| Separation time (min) | EPA content of the fraction (%) | Separation time (min) | EPA content of the fraction (%) |
| 20 | 13.02 | 12 | 9.11 |
| 21 | 20.05 | 13 | 4.15 |
| 23 | 34.82 | 14 | 10.06 |
| 24 | 47.25 | 15 | 21.46 |
| 25 | 62.5 | 15.5 | 29.13 |
| 26 | 71.44 | 16 | 40.14 |
| 26.5 | 74.5 | 16.5 | 54.65 |
| 27 | 76.22 | 17 | 63.67 |
| 27.5 | 77.39 | 17.5 | 68.77 |
| 28 | 78.59 | 18 | 73.71 |
| 29 | 79.85 | 19 | 78.4 |
| 30 | 80.05 | 19.5 | 80.8 |
| 31 | 79.39 | 20 | 81.48 |
| 32 | 76.37 | 21 | 80.31 |
| 33 | 74 | 22 | 76.77 |
| 34 | 70.8 | 23 | 72.49 |
| 35 | 68.48 | 24 | 69.63 |
| 36 | 66.17 | 25 | 66.77 |
| 37 | 64.6 | 26 | 64.32 |
| 38 | 64 | 27 | 66.69 |
| 39 | 62.62 | 28 | 60.73 |
| 40 | 61.82 | 29 | 59.48 |
| 41 | 60.99 | 30 | 58.03 |
| 42 | 60.62 | 32 | 54.65 |
| 44 | 59.23 | 34 | 51.4 |
| 46 | 57.65 | 36 | 46.5 |
| 48 | 56.66 | 38 | 42.34 |
| 50 | 55.32 | 40 | 37.86 |
| 52 | 53.51 | 45 | 27.81 |
| 55 | 48.97 | | |
| 60 | 44.15 | | |

Table 5

| EPA/DPA content in % in the case of separation using liquid carbon dioxide | | EPA/DPA content in % in the case of separation using supercritical carbon dioxide | |
|---|---|---|---|
| Separation time (min) | EPA/DHA content of the fraction (%) | Separation time (min) | EPA/DHA content of the fraction (%) |
| 20 | 13.02/1.69 | 12 | 9.11/26.52 |
| 21 | 20.05/0.92 | 13 | 4.15/3.59 |
| 23 | 34.82/0.48 | 14 | 10.06/1.12 |
| 24 | 47.25/0.65 | 15 | 21.46/0.45 |
| 25 | 62.5/0.92 | 15.5 | 29.13/0.41 |
| 26 | 71.44/1.38 | 16 | 40.14/0.39 |
| 26.5 | 74.5/1.63 | 16.5 | 54.65/0.52 |
| 27 | 76.22/1.93 | 17 | 63.67/0.73 |
| 27.5 | 77.39/2.19 | 17.5 | 68.77/0.97 |
| 28 | 78.59/2.54 | 18 | 73.71/1.43 |
| 29 | 79.85/3.42 | 19 | 78.4/2.23 |
| 30 | 80.05/4.59 | 19.5 | 80.8/2.68 |
| 31 | 79.39/6.21 | 20 | 81.48/3.21 |
| 32 | 76.37/8.36 | 21 | 80.31/5.23 |
| 33 | 74./1.88 | 22 | 76.77/8.96 |
| 34 | 70.8/15.08 | 23 | 72.49/13.52 |
| 35 | 68.48/17.73 | 24 | 69.63/16.38 |
| 36 | 66.17/19.53 | 25 | 66.77/19.32 |
| 37 | 64.6/21.21 | 26 | 64.32/22.11 |
| 38 | 64/22.2 | 27 | 66.69/20.21 |
| 39 | 62.62/23.39 | 28 | 60.73/25.81 |
| 40 | 61.82/24.35 | 29 | 59.48/27.33 |
| 41 | 60.99/24.8 | 30 | 58.03/28.62 |
| 42 | 60.62/25.12 | 32 | 54.65/31.5 |
| 44 | 59.23/26.89 | 34 | 51.4/34.19 |
| 46 | 57.65/28.31 | 36 | 46.5/38.47 |
| 48 | 56.66/29.39 | 38 | 42.34/42.23 |
| 50 | 55.32/30.58 | 40 | 37.86/45.76 |
| 52 | 53.51/31.95 | 45 | 27.81/53.76 |
| 55 | 48.97/34.7 | | |
| 60 | 44.15/38.6 | | |

Abbreviations used in the text:

| | |
|---|---|
| HPLC | high performance liquid chromatography |
| SFC | chromatography using supercritical gas |
| SbFC | chromatotgraphy using fluid gas ($T<T_{kr}$; $p>p_{kr}$) |
| LFC | chromatography using liquefied gas ($T<T_{kr}$; $p<p_{kr}$) |
| EPA | ethyl eicosapentaenoate (20:5) |
| DHA | docosahexaenoic acid (22:6) |